

PATENT COOPERATION TREATY

1FD

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

Subject to PTA? **YES**
per docket/ECB

To:

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PCT

WRITTEN OPINION

(PCT Rule 66)

DOCKETED

MAY 15 2002

6-12-02

Date of mailing
(day/month/year)

08.05.2002

Applicant's or agent's file reference

M0656/7063WO

REPLY DUE

within 2 month(s)
from the above date of mailing

International application No.

PCT/US01/07464

International filing date (day/month/year)

08/03/2001

Priority date (day/month/year)

08/03/2000

International Patent Classification (IPC) or both national classification and IPC

C12N15/60

Applicant

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

1. This written opinion is the ^{second} ~~first~~ drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain document cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

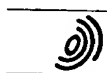
Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 08/07/2002. ✓

7 days
7/15/02

Name and mailing address of the international preliminary examining authority:



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WRITTEN OPINION

International application No. PCT/US01/07464

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"):

Description, pages:

1-69 as originally filed

Claims, No.:

1-60 as originally filed

Drawings, sheets:

1/17-17/17 as originally filed

Sequence listing part of the description, pages:

1-3, filed with the letter of 11.04.01

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

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International application No. PCT/US01/07464

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 28-60,

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 28-60.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

- ☐ restricted the claims.

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- ☐ paid additional fees.
 - ☐ paid additional fees under protest.
 - ☒ neither restricted nor paid additional fees.
2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
see separate sheet
3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:
- ☐ all parts.
 - ☒ the parts relating to claims Nos. 1-27.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement
- | | | |
|-------------------------------|--------|---|
| Novelty (N) | Claims | 1-5, 9, 11-14, 16-17 and 23-27 (NO) |
| Inventive step (IS) | Claims | 1-17 and 23-27 (NO) |
| Industrial applicability (IA) | Claims | 4-6, 9, 11, 15 and 20-22 (completely) and 1-3, 8, 10, 12-14 and 16 (partially) (see Citations and explanations) |
2. Citations and explanations
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

A unity objection was raised by the International Search Authority (ISA). Since the applicant didn't pay additional search fees, only invention 1 (claims 1-27) has been searched and, therefore, examined.

Re Item IV

Lack of unity of invention

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The IPEA agrees with the ISA advices that the present application lacks unity and identifies the following groups of inventions in the international application :

1. Claims : 1-27

Methods for preventing proliferation of a tumor or for preventing tumor cell metastasis, comprising exposing a tumor cell to heparinase III, either native or modified.

Methods for preparing therapeutic agents, i.e. HLGAG fragments, for tumor treatment comprising isolating of a portion of a tumor, treating it with heparinase III to produce HLGAG fragments, and isolating HLGAG fragments, possibly further comprising determining the sequence of the HLGAG fragments.

Methods for treating a subject having a tumor, comprising administering to the subject a therapeutic, synthetic or isolated HLGAG fragment, identified or produced when the tumor is contacted with heparinase III.

Pharmaceutical compositions comprising a therapeutic HLGAG fragment for preventing metastasis of a tumor cell, e.g. with an anti-cancer compound.

2. Claims : 28-49

A substantially pure heparinase III comprising a polypeptide according to SEQ ID NO:2, or having conservative substitutions thereof within residues non-essential to enzymatic function wherein at least one His residue from the group His36, His105, His110, His139, His152, His225, His234, His241, His424, His469 and His539 has been substituted with an Ala, Ser, Tyr, Thr or Lys residue.

A substantially pure heparinase III having a modified product profile which is at least 10% different than the product profile of native heparinase III.

A substantially pure heparinase III that can cleave a heparan sulfate having a modified k-cat value which is at least 10% different than a k-cat value of native heparinase III.

A pharmaceutical preparation comprising heparinase III as said.

An immobilized substantially pure modified heparinase III comprising a modified heparinase III as said and a solid support.

A method of specifically cleaving a HLGAG comprising contacting a HLGAG with a modified heparinase III as said. e.g. wherein the heparinase III is administered to subject in need for inhibiting angiogenesis, or wherein the heparinase is administered to a tumor, or wherein the heparinase III is administered in a polymeric delivery device or in a vehicle for injection or in a vehicle for topical application (e.g. to the eye), or wherein the method is a method for sequencing HLGAG fragments.

3. Claims : 50-54

Methods for treating or preventing a subject having a cancer or at risk of developing a cancer, comprising administering a therapeutic HLGAG fragment, e.g. a composition of HLGAG fragments wherein at least 50%, 75% or 90% of the HLGAG fragments are di- or tri-sulfated disaccharides, or wherein the therapeutic HLGAG fragment is free of mono- or unsulfated disaccharides.

4. Claims : 55-60

A method for preparing LMWH comprising contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

A composition comprising LMWH produced by contacting an HLGAG sample with a modified heparinase III. Methods of treating or preventing, e.g. of a disorder associated with coagulation, or of a tumor, or of psoriasis, or of neovascularization comprising administering to a subject a composition as said.

The prior art contains the following documents :

- WO9513830 disclosing and claiming the inhibitory effect of heparinase on angiogenesis, e.g. in a tumor.
- WO9201003 disclosing and claiming glycosaminoglycan derivatives and their use, e.g. in impeding the formation of tumor metastases.
- R. Godavarti et al. (1996) Biochem. Biophys. Res. Commun. 225 : 751-758 and WO9412618 disclosing heparinase III and its encoding gene from Flavobacterium heparinum
- EP0244236 and EP0394971 disclosing the preparation of a low-molecular weight heparin (LMWH) by chemical or enzymatic degradation of heparin or heparan sulfate, e.g. with the help of heparinase, and its use in inhibition of angiogenesis and the treatment of tumors.

In the light of these prior art documents, a first problem underlying this application can be defined as the need for further means and methods for preventing proliferation of a tumor or for preventing tumor cell metastasis. The solution as disclosed and claimed can be summarized as the provision of such means and methods comprising compositions containing heparinase III, or therapeutic heparin-like glycosaminoglycan (HLGAG) fragments obtained with the help of heparinase III and methods for the preparation and sequencing of these HLGAG fragments comprising the use of heparinase III, as well as the use of these compositions.

A second problem underlying the current application in view of the prior art can be summarized as the need for further heparinases. The solution as disclosed and claimed can be summarized as the need for further heparinases. The solution as disclosed and

claimed can be summarized as the provision of native or modified forms (mutants) of heparinase III, and the uses of these modified heparinases III.

A third problem underlying the current application in view of the prior art can be summarized as the need for further methods for the preparation of LMWH. The solution as disclosed and claimed can be summarized as the provision of a method for preparing LMWH comprising the use of a modified heparinase III, as well as the use of said LMWH in the preparation of pharmaceutical compositions and their use in treating or preventing disorders and diseases.

In view of the fact that methods for treating or preventing tumor proliferation or metastasis, e.g. comprising administering heparinases, glycosaminoglycans or LMWH are known, and methods for their preparation are known, in view of the different problems underlying the different solutions as disclosed and claimed, and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical feature common to these solutions, the IPEA agrees with the ISA advices that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is a lack of unity and the groups mentioned above represent independent inventions.

The attention of the applicant is drawn to the fact that claims 1-27 further lack unity for the following reasons :

1. Claims 1-17 refer to methods for preventing tumor cell proliferation or metastasis by treating said cells with heparinase III.
2. Claims 18-19 refer to a method for preparing therapeutic HLGAG fragments for the treatment of a tumor by treating a portion of a tumor with heparinase III.
3. Claims 20-22 refer to methods of treatment of a subject having a tumor by administering a therapeutic HLGAG fragment to said subject.
4. Claims 23-27 refer to compositions comprising a heparinase III and a targeting molecule or a therapeutic HLGAG fragment.

The use of heparinase III (optionally linked to a targeting molecule) for preventing cancer being well known from documents D1 and D4 (see points V-3.1 and V-4 of the present opinion) and HLGAG fragments produced by heparinase III being well-known from documents D2 and D3 and the targeting (see points V-3.1 and V-3.2 of the present opinion), the IPEA fails to see what could be considered as an inventive common concept linking the different groups mentioned above. Therefore, the present application lacks unity and the different groups mentioned above represent independent inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to methods for preventing proliferation of a tumor or for preventing tumor cell metastasis comprising exposing a tumor cell to an effective amount of (optionally modified) heparinase III, optionally in association with additional anti-cancer drugs. The application also refers to a method for preparing therapeutic agents (HLGAG fragments) for the treatment of a tumor and to compositions comprising heparinase III or therapeutic HLGAG fragments and a targeting molecule for targeting heparinase III to the tumor.

2. **Reference is made to the following documents :**

D1: WO 96 01648 A (IBEX TECHNOLOGIES R AND D INC ;ZIMMERMANN JOSEPH (US); VLODAVSKY I) 25 January 1996 (1996-01-25)

D2: WO 94 21689 A (CANCER RES CAMPAIGN TECH ;LYON MALCOLM (GB); GALLAGHER JOHN THOMAS) 29 September 1994 (1994-09-29)

D3: WO 93 19096 A (CANCER RES CAMPAIGN TECH) 30 September 1993 (1993-09-30)

D4: WO 95 13830 A (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 26 May 1995 (1995-05-26)

3. **Lack of novelty; article 33(2) PCT.**

3.1 The document D1 discloses, inter alia, the fact that selectively removing

heparan sulphate from cell surfaces while leaving the extracellular matrix intact inhibits cell proliferation by down regulating the cell's response to growth factors and that this can be achieved by targeting heparin or heparan sulphate degrading activities to the cell surface. The glycosaminoglycan degrading enzymes comprise heparinase I, II and III. The targeting of said enzymes can be achieved by genetically engineering a ligand binding functionality into heparinase proteins or by physically controlling the localized enzyme concentration through the method of administration. (Abstract). Means of administration are disclosed on p.32 and include topically administration or injection.

Claim 8 discloses a method to diminish the response of a cell to growth factors by directing a glycosaminoglycan degrading enzyme to the surface of targeted cells. Claim 9 specifies that targeting is achieved by incorporating a cell specific ligand binding function and heparin or heparan sulfate degrading activity into a fusion protein having glycosaminoglycan degrading enzyme activity and claim 11 specifies that the ligand is specific to or present in higher concentration in cancer cells as compared with normal cells. Claims 17-28 refer to pharmaceutical compositions comprising a glycosaminoglycan degrading enzyme in combination with a pharmaceutically acceptable carrier and optionally means for targeting the enzyme to cells or tissues (claims 23-25).

Even if the mechanism of action of the heparinase III was not known from the authors of D1, the method disclosed in D1 is the same as the method disclosed in the present application, i.e. targeting a heparinase - which can be heparinase III - to a tumor cell. Since no further indications are given in the methods of the present application, the IPEA assumes that the mere presence of heparinase III at the surface of a tumor cell will be sufficient to prevent proliferation of the tumor cell or metastasis.

Moreover, the IPEA is the opinion that the fusion protein disclosed in D1 can be considered as a modified heparinase III.

Therefore, the subject-matter of claims 1-5, 9, 11-14 and 16-17 can not be considered as novel in the sense of article 33(2) PCT.

The attention of the applicant is drawn to the fact that even if the specific use for the compositions of D1 is not given, some of the compositions claimed in D1 will have the same components as the compositions claimed in the present application. Therefore, claims 23-26 can not be considered as novel in the sense of article 33(2) PCT.

- 3.2 The document D2 discloses heparan sulphate oligosaccharides having hepatocyte growth factor affinity. The oligosaccharides disclosed in D2 are composed of at least three disaccharide units including one or more internal sequences of an N-sulphated D-glucosamine 6-sulphate residue and an L-iduronic acid residue (Abstract). The oligosaccharide chains disclosed in D1 are obtained by enzymatic partial depolymerisation to the fullest extent of heparan sulphate using heparinase III, followed by size fractioning (p. 5, last paragraph). D2 also claims such an oligosaccharide composition (claims 1-19), methods of isolating low molecular weight oligosaccharides from heparan sulphate proteoglycans of mammalian cells using heparinase III treatment (claim 28) and the therapeutic use of the oligosaccharide compositions (claims 30-33).

The IPEA is the opinion that even if the compositions of D2 have not been specifically disclosed for use in treatment of cancer, the compositions disclosed in D2 will be the same as the compositions disclosed in claims 23-27 as far as they refer to HLGAG fragments. Therefore, claims 23-27 can not be considered as novel over the teaching of D2 (article 33(2) PCT).

- 3.3 The document D3 discloses oligosaccharides having growth factor binding affinity. These oligosaccharides can be prepared from glycosaminoglycans such as heparan sulphate and can be used either to activate and stimulate FGF activity or inhibit FGF activity. The use of said oligosaccharides for therapeutic purposes in medicine is also disclosed (Abstract). Some of the oligosaccharides were depolymerised using heparitinase (i.e. heparinase III) (p. 33-34, Depolymerisation of HS to selectively produce sulphated oligosaccharides). Further purifications steps are disclosed (p. 34-38). The use of the oligosaccharides for antitumour treatment is suggested (p. 40, lines 19-21). Claim 11 discloses an oligosaccharide product obtained by

digestion of heparan sulphate by heparitinase, claim 25 discloses a method for obtaining such an oligosaccharide product using heparitinase, claims 33-35 refer to the therapeutical use of such an oligosaccharide product, inter alia for inhibiting cancer cell growth and metastasis and claim 40 refers to a method of treatment, inter alia for inhibiting cancer cell growth and metastasis using an oligosaccharide product according to D3.

Therefore, the subject-matter of claims 23-27 as far as they refer to HLGAG fragments can not be considered as novel over the teaching of D3 (article 33(2) PCT) (see also points 3.1 and 3.2 for the explanations).

4. Lack of inventive step; article 33(3) PCT.

The IPEA is the opinion that the skilled person, knowing from the document D1 that tumor growth or metastasis could be prevented by targeting a heparinase (including heparinase III) to the surface of a tumor cell, would have contemplated to administrate the heparinase orally or to administrate said enzyme in conjunction with other well-known anti-cancer compounds.

Therefore, claims 6, 8 and 15 can not be considered as inventive (article 33(3) PCT).

The IPEA is also the opinion that the treatment of a tumor with heparinase in vitro can also not be considered as inventive. Therefore, claims 7 lacks inventive step in the sense of article 33(3) PCT.

The selection of specific tumor to be treated can also not be considered as inventive as long as this selection is not motivated by a technical purpose. For the moment, the IPEA fails to see such a technical purpose for the selection of the prostate tumor and melanoma. Therefore, claim 10 can not be considered as inventive (article 33(3) PCT).

The attention of the applicant is also drawn to the fact that the document D4 deals with the use of heparinases - including heparinase III - for inhibiting angiogenesis. An application of the methods disclosed in D4 is the treatment of solid tumors. Different ways of administration of heparinase are discussed, including direct

injection in tumors (p. 38-39).

Even if the purpose for administering heparinase III in D4 (inhibiting angiogenesis which is necessary for solid tumor growth and metastasis) is not the same as in the present application, the administration of said heparinase to tumor cells for treating cancer has been suggested in D4. Therefore, the subject-matter of claims 1-17 can not be considered as inventive over the teaching of D4 (article 33(3) PCT).

5. Industrial applicability; article 33(4) PCT.

Claims 4-6, 9, 11, 15 and 20-22 refer to method of treatment of the human or animal body.

The methods of claims 1-3, 8, 10, 12-14 and 16 (partially) can be considered as method of treatment of the human or animal body as long as they are practised in vivo.

For the assessment of the present claims 4-6, 9, 11, 15 and 20-23 (completely) and claims 1-3, 8, 10, 12-14 and 16 (partially) on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

6. Further remarks concerning the application.

6.1 The attention of the applicant is drawn to the fact that, in the light of the prior art, the composition of the HLGAGs at the surface of a cell can greatly vary from one cell type to another. Therefore, the IPEA considers that it is questionable if the HLGAG fragments resulting from heparinase III treatment of every type of tumor cell will result in the generation of HLGAG fragments having a tumor- or metastasis-preventing activity according to the present application.

6.2 Claims 23-27 refer to compositions comprising, inter alia, HLGAG fragments. The IPEA is the opinion that said claims are not clear for the following reasons :

- (i) All HLGAG fragments - including those generated by heparinase I or II, for example - will not have an effect for preventing tumor growth or metastasis. The IPEA is the opinion that, faced to all the possible HLGAG fragments covered by claims 23-27, the skilled will not be able to determine those having a tumor growth or metastasis-preventing activity without undue burden of experimentation.
- (ii) The attention of the applicant is drawn to the fact that the HLGAG fragments, being products, should be defined in terms of technical features of the product. The IPEA is the opinion that the definition of the HLGAG fragments in term of process steps would also not be considered as sufficiently clear since the method for producing HLGAG fragments in the present application will give very different HLGAG fragments, depending on the nature of the HLGAG at the surface of the cell and the conditions of the heparinase III treatment.
- (iii) The compositions of claims 23-27 comprise heparinase III and a targeting molecule. However, in said claims, nothing is said about the link between heparinase III and the targeting molecule. The IPEA is the opinion that the mere presence of a targeting molecule in the compositions of claims 23-27 is not sufficient to target the heparinase III to a specific site.